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## ARTICLES

# In vitro cleavage of hepatitis C virus polyprotein substrates by purified recombinant NS3 protease

ED D'Souza, K Grace, DV Sangar, DJ Rowlands and BE Clarke

Biology Division, Wellcome Research Laboratories, Beckenham, Kent, UK.

The non-structural protein NS3 of hepatitis C virus has been expressed in bacteria as a polyhistidine fusion protein which can be produced in a soluble form and easily purified by affinity chromatography. Using an in vitro transcription and translation system we have been able to demonstrate that this protein can proteolytically process substrate molecules derived from the non-structural region of the polyprotein. Using this assay system we have been able to optimize basic biochemical characteristics of the purified enzyme. Parallel experiments show that the full-length NS3 protein also possesses ATPase activity, indicating the bifunctional nature of the protein. In contrast, purified NS3 in which the predicted catalytic serine has been mutated loses protease but retains ATPase activity.

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## ARTICLES

### In vivo and in vitro trans-cleavage activity of hepatitis C virus serine proteinase expressed by recombinant baculoviruses

T Suzuki, M Sato, S Chieda, I Shoji, T Harada, Y Yamakawa, S Watabe, Y Matsuura and T Miyamura

Department of Virology II, National Institute of Health, Tokyo, Japan.

By the use of recombinant baculoviruses, the trans-cleavage of hepatitis C virus (HCV) non-structural polyprotein was studied. The viral serine proteinase encoded by the NS3 gene was expressed efficiently in insect cells infected with a baculovirus recombined with HCV cDNA corresponding to amino acids 1046-1243 and the signal sequence of the rabies virus G protein. Coinfection studies showed the in vivo trans-cleavage activity of the expressed protein by the use of a recombinant producing NS5 as a substrate. We also found that the partially purified NS3 serine proteinase prepared from the recombinant- infected cells could cleave NS5A/5B substrate. Characterization of the proteinase obtained wil provide basic knowledge on processing of the HCV polyprotein.

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fusion

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